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(54) Title: METHOD TO USE SUPERANTIGENS TO TARGET SUBPOPULATIONS OF T CELLS

(57) Abstract

The invention describes a method to treat diseases mediated by pathogenic T cells, the majority of which express surface receptors comprised of one or few Vβ regions, by using a superantigen to mark T cells expressing these Vβ regions for inactivation or deletion by a cytotoxic agent. This method does not affect T cells expressing surface receptors comprised of other Vβ and, thus, leaves the overall immune system intact. The invention may be used, for example, in the treatment of autoimmune diseases.

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METHOD TO USE SUPERANTIGENS TO TARGET SUBPOPULATIONS OF T CELLS

FIELD OF INVENTION

This invention relates to the field of immunology. Specifically, this invention relates to the use of superantigens to specifically target pathogenic subsets of T cells for subsequent inactivation or deletion by an additional agent or agents. This application, thus, describes a method for treating diseases mediated by T cells having a limited Vβ profile by inactivating or deleting certain Vβ defined subpopulations of T cells which comprise the pathogenic T cells.

BACKGROUND OF THE INVENTION

The vertebrate immune system evolved to provide specific protection from infection by foreign micro- and macroorganisms. The immune system responds to antigens in one of two ways: (1) humoral antibody responses, mediated through B lymphocytes, or B cells, and (2) cell-mediated immune responses, mediated through T lymphocytes, or T cells. The B cell response results in the production of antibodies that circulate in the bloodstream and bind specifically to the foreign antigen that induced them. The binding of the antibody to the antigen either directly inactivates the antigen, marks it for ingestion by phagocytic cells or marks it for destruction by cytotoxic cells. It is now known that for most antigens the production of antibodies by B cells requires T cell help. The T cell response results in the activation and proliferation of antigen-specific T cells that, in addition to helping B cells, react directly with foreign antigens on the surface of host cells, either killing the host cell if the antigen is an infecting virus or inducing other host cells, such as macrophages, to destroy the antigen (Nossal (1993) Sci. Amer. **269**:52; Janeway (1993) Sci. Amer. **269**:72; Marrack and Kappler (1993) Sci. Amer. **269**:80).

This invention is concerned primarily with T cell immunity. In particular, this application describes a method for dealing with T cell responses

which have gone awry resulting in, for example, autoimmune diseases. The attack by the immune system on host cells can result in a large number of disorders, including neural diseases such as multiple sclerosis and myasthenia gravis, diseases of the joints such as rheumatoid arthritis, attacks on nucleic acids as observed with systemic lupus erythematosus, and diseases associated with various organs such as psoriasis, juvenile onset diabetes, Sjögren's disease and thyroid disease.

T cells have antigen-specific receptors on their surfaces, termed T cell antigen receptors (TCR). Before T cells can recognize protein antigens, the antigens must be presented on the surface of antigen-presenting cells, after macrophages or other antigen presenting cells have first processed the antigens. These cells essentially ingest antigens and degrade them into peptides which are displayed at the cell surface in combination with major histocompatibility complex (MHC) molecules (Shimonkevitz *et al.* (1983) J. Exp. Med. **158**:303; Babbit *et al.* (1985) Nature **317**:359).

The major histocompatibility antigens are a family of molecules encoded for by a related group of genes encoded within the major histocompatibility complex. MHC antigens are expressed on the cells of all higher vertebrates. In man they are called HLA antigens (human-leukocyte-associated antigens) because they were first demonstrated on leukocytes. There are two principal classes of MHC molecules, class I and class II, each comprising a set of cell-surface glycoproteins. The two classes of MHC antigens stimulate different subpopulations of T cells. MHC class II molecules are involved in most responses to extracellular pathogens, while MHC class I molecules are involved when the pathogen is cell-associated, i.e., when a virus or a malignant cell is involved. When MHC class I is involved, antibody stimulation does not result; rather, the interaction of MHC class I processed antigen and T-cell leads to lysis of cells infected with the pathogen.

Processed antigen peptide fits in a cleft on an MHC molecule (Bjorkman *et al.* (1987) Nature **329**:506). Once an antigen is displayed, the few T cells in the body that bear receptors for that particular peptide bind that complex. Most T cells recognize antigens on the surface of cells only in association with self-MHC glycoproteins expressed on the cell surface.

The ability of the T cell to bind to the processed antigen and MHC complex is dependent on the T cell receptor (TCR). The TCR consists of two protein chains, usually α and β chains. Each chain is composed of a constant and a variable domain. The variable domains are encoded in two (α) or three (β) different gene segments (variable (V), diversity (D), joining (J)) (Siu *et al.* (1984) Cell **37**:393; Yanagi *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:3430). In each T cell, the combination of V, D and J domains of both the α and β chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell and defines a unique binding site. (*See*, Marrack *et al.* (1988) Immunol. Today **9**:308; Toyonaga *et al.* (1987) Ann. Rev. Immunol. **5**:585; Kronenberg (1985) Ann. Rev. Immunol. **4**:529; Hedrick *et al.* (1982) Cell **30**:141). Generally, both the α and β chains are involved in recognition of the ligand formed by processed antigen and MHC.

When T cells are stimulated by an antigen, they divide and differentiate into activated effector cells that are responsible for various cell-mediated immune reactions. At least three different reactions are carried out by T cells: (1) cytolytic T cells specifically kill foreign or virus-infected vertebrate cells; (2) helper T cells help B lymphocytes; and (3) suppressor T cells suppress the responses of specific cells.

It has been shown that a novel class of antigens, termed "superantigens" (SAg), are able to directly stimulate T cells by binding to a particular subset of Vβ elements. That is, the variable domain of the β chain of the TCR alone determines whether or not a particular superantigen will stimulate a

particular T cell (Kappler *et al.* (1987) Cell **49**:263; Kappler *et al.* (1987) Cell **49**:273; MacDonald *et al.* (1988) Nature **332**:40; Pullen *et al.* (1988) Nature **335**:796; Kappler *et al.* (1988) Nature **332**:35; Abe *et al.* (1988) J. Immunol. **140**:4132; White *et al.* (1989) Cell **56**:27; Janeway *et al.* (1989) Immunol. Rev. **107**:61; Bekoff *et al.* (1988) J. Immunol. **139**:3189; Kappler *et al.* (1989) Science **244**:811). Unlike recognition of conventional peptide antigens, the other components of the T cell receptor (i.e., Dβ, Jβ, Vα, Jα) appear to play little role in the superantigen binding. Since the relative number of Vβ genes is limited, many T cells within an individual will bear a particular Vβ element, and a given superantigen is therefore capable of interacting with a large fraction of the total T cell repertoire. Thus, depending upon the frequency of the responding Vβ population or populations, 5-30 % of the entire T cell repertoire could be stimulated by a superantigen; whereas the responding frequency to a conventional antigen is usually much less than 1 in 1,000, or less than 0.1 %. Although superantigens interact with class II MHC molecules, they appear to act as intact proteins rather than as peptides. That is, they do not require processing for stimulation and they bind outside the conventional peptide binding groove (Jardetzky *et al.* (1994) Nature **368**:711; Seth *et al.* (1994) Nature **369**:324). They appear to interact with amino acid residues that are on the outer walls of the binding cleft. Known superantigens and references to their sequences and structures are listed in Table I.

Two distinct classes of superantigens have been described. The first was noted over 20 years ago, when Festenstein showed marked responses in mixed lymphocyte reactions between certain MHC identical strains. The stimulating antigens were called minor lymphocyte stimulating (Mls) antigens (Festenstein (1973) Transplant Rev. **15**:62) to differentiate them from MHC antigens. At that time, their superantigen character was not known. It is now known that these superantigens are encoded by endogenous retroviral genes (Palmer (1991) Curr.

Bio. 1:74). The presence of these genes in the mouse leads to a marked deletion of responding T cells, creating potentially large holes in the animal's T cell receptor repertoire (Pullen *et al.* (1988) *supra*). The second class of superantigen is represented by a growing list of bacterial and viral proteins, capable of producing a variety of pathological effects after injection (Marrack and Kappler (1990) Science **248**:705).

Staphylococcus aureus (*S. aureus*), a common human pathogen, produces several enterotoxins, designated as SEA (Staphylococcal enterotoxin A) through SEE (Staphylococcal enterotoxin E), which can be responsible for food poisoning and occasionally shock in humans (Marrack & Kappler (1990) *supra*; Bohach *et al.* (1990) Crit. Rev. Microbio. **117**:251). Some *S. aureus* isolates also produce toxic shock syndrome toxin-1 (TSST-1), which has been implicated in the majority of cases of human toxic shock syndrome, as well as the related exfoliative toxin (ExT), which is associated with the scalded skin syndrome. *Streptococcus pyrogenes*, or group A streptococcus, another common human pathogen of the skin and pharynx, also produces toxins with superantigenic properties (Abe *et al.* (1991) J. Immun. **146**:3747). These toxins have been designated Streptococcal erythrogenic toxins A-C (SPEA-C).

The amino acid sequences of the *S. aureus* toxins exhibit some homology, but also exhibit marked differences (*See*, Betley *et al.* (1988) J. Bacteriol. **170**:34; Jones *et al.* (1986) J. Bacteriol. **166**:29; Couch *et al.* (1988) J. Bacteriol. **170**:2954; Blomster-Hautamaa *et al.* (1986) J. Biol. Chem. **261**:15783). *S. aureus* toxins have the ability to stimulate powerful T cell proliferation responses in the presence of mouse cells bearing MHC class II type molecules (White *et al.* (1989) *supra*). The *S. aureus* proteins selectively stimulate murine and human T cells bearing particular Vβ elements.

The binding of toxins to class II MHC molecules is usually required for T cell recognition. However, the process is much more permissive for

superantigens than that seen with conventional antigens. While peptide antigens are very dependent on allelic MHC residues for binding, superantigens bind to a wide variety of allelic and isotypic forms of MHC class II molecules (*See*, Herrmann *et al.* (1989) Eur. J. Immunol. **19**:2171; Herman *et al.* (1990) J. Exp. Med. **172**:709; Scholl *et al.* (1990) J. Immunol. **144**:226; Mollick *et al.* (1991) J. Immunol. **146**:463). Whereas T cells rarely recognize peptide antigens bound to self-MHC molecules, individual T cell clones can respond to toxins bound not only to various allelic forms of MHC but also to different class II isotypes and even xenogeneic class II molecules. Observations such as these reinforce the concept that superantigens bind at a relatively conserved site outside the allelically variable groove where conventional peptide antigens bind.

Superantigens may contribute to autoimmune diseases in which components of the immune system attack normal tissue. The process of deletion of T cells responsive to self, potentially harmful self-reactive T cells, is called tolerance or negative selection (Kappler *et al.* (1987) *supra*; Kappler *et al.* (1988) *supra*; Von Boehmer *et al.* (1988) Immunol. Rev. **101**:21). The immune system usually deletes self-reactive T cells, with good, but not complete efficiency, thus, some self-reactive cells appear to escape the surveillance mechanism. It is possible that the ability of superantigens to arouse 20 percent of a person's T cell repertoire leads to undesirable replication of the few circulating T cells that are capable of recognizing self (Kotzin *et al.* (1993) Adv. Immunol. **54**:99). T cells bearing certain Vβ types have been implicated in various autoimmune conditions, such as arthritis and multiple sclerosis. These destructive cells might have been initially activated by a superantigen that binds to the identified Vβ types (Kotzin *et al.* (1993) *supra*), leading to disease.

Several lines of evidence suggest that T cells specific for self-antigens play a critical role in the initiation of autoimmune diseases. In the case of rheumatoid arthritis, the linkage of the disease to the DR4 and DR1 alleles of the

class II genes of MHC, and the finding of oligoclonal activated $CD4^+$ T cells in synovial fluid and tissue of affected joints (Stastny *et al.* (1976) Engl. J. Med. **298**:869; Gibofsky *et al.* (1978) J. Exp. Med. **148**:1728; McMichael *et al.* (1977) Arth. Rheum. **20**:1037; Schiff *et al.* (1982) Ann. Rheum. Dis. **41**:403; Duquestoy *et al.* (1984) Hum. Immunol. **10**:165; Legrand *et al.* (1984) Am. J. Hum. Genet. **36**:690; Gregerse *et al.* (1987) Arth. Rheum. **32**:15; Burmester *et al.* (1981) Arth. Rheum. **24**:1370; Fox *et al.* (1982) J. Immunol. **128**:351; Hemler *et al.* (1986) J. Clin. Invest. **78**:696; Stamenkoic *et al.* (1988) Proc. Natl. Acad. Sci. USA **85**:1179; Paliard *et al.* (1991) Science **253**:325) suggest the involvement of $CD4^+$, α/β TCR-bearing, class II-restricted T cells in the disease. This view is supported by the finding that partial elimination or inhibition of T cells by a variety of techniques can lead to an amelioration of disease in certain patients (Paulus *et al.* (1977) Arth. Rheum. **20**:1249; Karsh *et al.* (1979) Arth. Rheum. **22**:1055; Kotzin *et al.* (1989) N. Eng. J. Med. **305**:976; Herzog *et al.* (1987) Lancet **ii**:1461; Yocum *et al.* (1989) Ann. Int. Med. **109**:863).

Although the mechanism underlying the association of particular MHC alleles with autoimmune disease is not yet fully understood, it has been well demonstrated that $CD4^+$ T cells recognize a complex consisting of an MHC class II molecule and a bound protein fragment (peptide) of about 15 amino acids. It is commonly accepted that the ability of a particular MHC molecule to bind particular peptides underlies its association with an increased or decreased propensity for disease. Current therapy for most autoimmune diseases consists of non-specific immune suppression (by the administration of, e.g., cyclosporin, steroids) or of non-specific elimination of rapidly dividing cells (by the administration of, e.g., cytoxan, methotrexate). In both cases, treatment is limited by the deleterious effect of immune suppression or toxicity to non-immune cells such as myeloid and epithelial cells.

In treating autoimmune diseases, it is preferable to liminate only those T cells that cause or perpetuate the disease; thus, leaving the immune system intact and non-immune cells unaffected. T cells responsible for disease differ from beneficial T cells by their TCR. The initial problem then, is to identify those T cells bearing aberrant or pathogenic T cell receptors. Assuming all receptors have an equal likelihood of causing disease, identifying those responsible is a formidable task. However, MHC associations with particular diseases suggest that the actual number of pathogenic TCRs may be small. The most straight forward model for this association is that the implicated MHC molecule binds one of a few peptides derived from newly available self or foreign antigens and that this MHC-peptide complex stimulates T cells that either react or cross-react with self-proteins and cause disease. It is unlikely that a large number of peptides would bind exclusively to a single MHC molecule. Indeed if this were the case, one would not expect to see any MHC association with a particular disease. When the T cell response of inbred mice to a specific peptide presented by a particular MHC molecule is studied, the general finding is that only a few TCRs are involved and those involved share expression of one or a few Vα and/or Vβ gene segments (Winoto *et al.* (1986) Nature **324**:679). In mice, where this phenomenon has been more extensively studied (e.g., EAE, collagen arthritic), the responsible T cells express a very limited T cell receptor repertoire. (Acha-Orbea *et al.* (1988) Cell **54a**:263; Urban *et al.* (1988) Cell **54**: 577; Chluba *et al.* (1989) Eur. J. Immunol. **19**:279; Osman *et al.* (1993) J. Exp. Med. **177**:387). In these cases the limited repertoire results from T cell recognition of only one or a few peptides derived from the incitatory antigen (e.g., MBP or collagen).

As previously discussed, SAgs were first discovered by their ability to cause deletion of T cells expressing particular Vβs. Subsequently, many investigators have deliberately employed SAgs in order to cause deletion or non-responsiveness of Vβ subsets of T cells (Rammensee *et al.* (1989) Nature **339**:541;

Webb *et al.* (1990) Cell **63**:1249; Blackman *et al.* (1990) Nature **345**:540; Jones *et al.* (1990) Science **250**:1726; Danecker *et al.* (1991) J. Immunol. **146**:2083; Ignatowicz *et al.* (1992) J. Exp. Med. **175**:917; Kawabe and Ochi (1990) J. Exp. Med. **172**:1065; Kawabe and Ochi (1991) Nature **349**:245; Rellahan *et al.* (1990) J. Exp. Med. **172**:1091; MacDonald *et al.* (1991) Eur. J. Immunol. **21**:1963; McCormick *et al.* (1993) J. Immunol. **150**:3785; Ochi *et al.* (1993) Semin. Immunol. 5:57). Generally, a stimulatory phase precedes the deletion/non-responsiveness induced by superantigens. During the stimulatory phase, in addition to T cell proliferation, a large quantity of cytokines is released. Since superantigens stimulate a larger portion of the total T cell population than do conventional antigens, such as viruses or bacteria, cytokines are released in quantities that can be toxic rather than beneficial. Toxic Shock Syndrome is an example of such toxicity (Bergdoll *et al.* (1981) Lancet **i**:1017; Schlievert *et al.* (1981) J. Infec. Dis. **143**:509; Todd (1988) Clin. Microbiol. Rev. 1:432; Chesney (1989) Rev. Infec. Dis. **11**:S1; Bohach *et al.* (1990) Crit. Rev. Microbiol. **17**:251).

U.S. Patent 5,336,598, entitled: *Method for Diagnosing a Superantigen caused pathological condition via assay of T cells* and U.S. Patent 5,298,396 entitled: *Method for Identifying T Cell Involved in Autoimmune Disease*, hereby specifically incorporated by reference, describe methods in which the identification of Vβ element levels and characterization of Vβ-specific T cell populations are indicative of superantigen-mediated pathological conditions. Previous approaches to achieve SAg mediated deletion of T cells while avoiding toxic side effects have included manipulations of SAg dose. U.S. Patent Application Serial No. 08/045,494, filed April 8, 1993, entitled: *Method of Treatment of Disease by Deletion of T Cells*, hereby specifically incorporated by reference, describes a method of treating superantigen-mediated diseases using repeated administration of low dose SAg.

Non-SAg mediated approaches deleting pathogenic subsets of T cells based on Vβ expression have also been proposed. For example, European Patent Publication 340,109, entitled *Anti-T-cell receptor determinants as autoimmune disease treatment*, and U.S. Patent No. 4,550,086, issued October 29, 1985 to Reinherz *et al.* entitled *Monoclonal antibodies that recognize human T cells*, describe a method of detecting a particular sequence of the variable region gene of T cell receptors associated with a particular disease and treating the disease with antibodies to that sequence. A method of diagnosing diseases based on the presence of T cells with a unique sequence in the Vβ region associated with a specific disease, a method for detecting specific Vβ regions associated with rheumatoid arthritis (RA), specifically, Vβ3, Vβ9 and Vβ10 and a method for the treatment of RA with monoclonal antibodies which recognize Vβ3, Vβ9 and Vβ10 have been described as well (U.S. Patent 4,886,743; PCT Patent application publication WO 90/06758).

One application for the present invention involves a treatment for rheumatoid arthritis. Although the specific findings vary, as discussed above, several investigators report oligoclonal expansions of T cells in patients with rheumatoid arthritis. In particular, a number of investigators implicate T cells expressing TCRs comprised of one or more of the Vβs targeted by the superantigen SEB--namely Vβs 3, 12, 14, 15 and 17 (Williams *et al.*(1992) J. Clin. Invest. **90(2)**:326; Williams *et al.* (1993) DNA Cell Biol. **12(5)**:425; Paliard *et al.* (1991) Science **253**:325; Howell *et al.* (1991) PNAS **88**:10921; Uematsu *et al.* (1991) PNAS **88**:8534; Maruyama *et al.* (1993) Eur. J. Immunol. **23(9)**:2059; Jenkins *et al.* (1992) J. Clin. Invest. **92(6)**:2688; Pluschke *et al.* (1993) Immunobiol. **188(4-5)**:330). Thus, reducing the responsiveness of or deleting T cells expressing the Vβs targeted by SEB may be therapeutic in Rheumatoid Arthritis.

SUMMARY OF INVENTION

This invention is based n a novel concept for the treatment of subject animals having certain diseases. The present invention is defined by the administration of a superantigen or superantigen derivative to mark certain T cell subpopulations based on their TCR Vβ expression for subsequent inactivation or deletion by an additional agent or agents.

The present invention is applicable to any disease caused by subpopulations of T cells, said subpopulations defined by the Vβ element which comprises their antigen receptor (TCR). The present invention is useful to treat certain autoimmune diseases, but is not intended to be limited to these diseases.

In one embodiment of the method of this invention, a superantigen or superantigen derivative is administered in order to mark a Vβ defined subpopulation of T cells that includes pathogenic T cells. As a result of superantigen activation via their TCR, the target subpopulation of T cells becomes phenotypically different from other T cells. As a result of SAg activation, the targeted T cells proliferate, produce cytokines, and express or up-regulate certain surface proteins. It is possible, then, to exploit these phenotypic changes in order to inactivate or delete the marked T cell subpopulation. Furthermore, it is possible to kill rapidly proliferating cells by the administration of cytotoxic agents such as methotrexate. For example, T cells which express Fas become susceptible to killing by Fas ligand.

The activation of certain Vβ subpopulations of T cells may result in release of toxic amount of cytokines. In another embodiment of the present invention the administration of an additional agent or agents is contemplated in order to prevent or ameliorate cytokine release.

Other features and advantages of the present invention will become apparent from the following detailed description, taken in conjunction with the

accompanying figures, that illustrate by way of example, the principles of the instant invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows tritiated thymidine incorporation by human peripheral blood lymphocytes (hPBLs) stimulated with toxins.

Figure 2 shows light scatter patterns of hPBLs with and without SEB stimulation.

Figure 3 shows an analysis of TCR expression in resting T cells versus proliferating T cells.

Figure 4 shows the cell viability and Vβ3 TCR expression over time, in culture.

Figure 5 shows the effect of methotrexate on SEB induced T cell blasts.

Figure 6 shows the deletion of Vβ3, 12 and 17 T cells by treatment with SEB and Methotrexate (MTX).

Figure 7 shows the dose of methotrexate required to prevent SEB induced T cell blasts.

Figure 8 shows the deletion of Vβ2 T cells by TSST-1 + methotrexate/dexamethasone.

Figure 9 shows the prevention of IL-2 and TNFα production in response to SEB by pre-administration of dexamethasone (DEX).

Figure 10 shows the ability of dexamethasone to prevent superantigen induced toxicity in mice.

Figure 11 shows a primate system to study SEB mediated T cell deletion.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

The present invention describes a method for the treatment of certain diseases and medical conditions. Medical conditions and diseases that may be treated according to the present invention are those that are induced or maintained, either in their inception or course of action, by subpopulations of T cells that are defined by the Vβ element which comprises their T cell antigen receptor (TCR).

It is well know that superantigens react with T cells in a Vβ specific manner. This is due to the fact that superantigens do not require processing for stimulation and they bind outside the conventional peptide binding groove. That is to say, superantigens interact with amino acid residues that are on the outer walls of the binding cleft but bind to the exterior of the MHC TCR pocket. Normal superantigens are capable, therefor, of activating a specific subset of TCRs. This feature may be taken advantage of in situations where the particular subsets of Vβs that are marked by a superantigen may also be implicated in the causation or mediation of a disease or medical condition.

In the broadest conception of the invention, the present invention specifies the administration of an agent, preferably a superantigen or superantigen derivative, capable of marking certain T cell subpopulations, based upon their TCR Vβ expression, for subsequent inactivation or deletion by the administration of an additional agent or agents. The superantigen or superantigen derivative administered acts to "mark" a given subset of Vβ containing T cells. Where the superantigen administered is not a superantigen derivative, the Vβs that are marked are those that are naturally associated with that superantigen. In such cases, the marking is generally the activation of the T cells that have been targeted. In other embodiments of the invention, where the superantigen administered is a

superantigen derivative, the TCR Vβs that are marked for subsequent inactivation or deletion include Vβs other than those naturally targeted by the unmodified superantigen. In addition, the marking of T cell subpopulations does not necessarily give rise to the activation of the T cells; marking in some other way may distinguish or target the selected subset of T cells so that they can be selectively inactivated or deleted by the agent or agents co-administered with the superantigen or superantigen derivative.

In the most preferred embodiment of the invention, a cytotoxic agent is administered along with the superantigen or modified superantigen that will selectively kill that T cells that have been marked by the superantigen. The cytotoxic agent will preferentially kill rapidly dividing activated T cells. In doing so, those selected T cell TCR Vβ subsets associated with the disease or medical condition are specifically killed, therefor acting as a method for the prevention or treatment of the disease or medical condition.

By way of example, the superantigen Staphylococcal enterotoxin B (SEB) activates T cells expressing TCRs comprised of Vβ 3, 12, 14, 15, 17 and 20. As a result of SEB induced activation, T cells expressing these Vβs will begin to proliferate and thus become susceptible to killing by cytotoxic agents. That is to say, SEB is used to kill certain T cells by causing them to proliferate in the presence of, for example, methotrexate. In this and other examples of the invention, neither the administration of a toxin nor the administration of a cytotoxic agent, in and of themselves, prevents cytokine release. Hence, an additional agent is needed to control potential toxicity. In one embodiment of the invention, the additional agent is an immunosuppressive agent, such as a corticosteroid.

Various terms are used in this specification, for which it may be helpful to have definitions. These are provided herein, and should be borne in mind when these terms are used in the following examples.

A key event in the immune response of an animal is the interaction of MHC molecules with antigens to form a complex presented to T cells.

"Animal" is defined as an organism of the kingdom Animalia, as distinguished from plants by certain typical characteristics. In the instant application, a subject animal is preferably a vertebrate, and more preferably a mammal, in whom it is desirable to modify the responsiveness of certain subpopulations of T cells.

The "antigens" generally recognized by T cells consist not of intact proteins but of peptide fragments bound to a binding groove in an MHC molecules. The T cell response to a particular antigen/MHC complex is highly specific and requires interaction of most or all of the components of both chains of the T cell receptor. Thus only a very small number of T cells respond to a given antigen.

"Superantigen" means a protein which upon binding to an MHC molecule is capable of interacting with subpopulations of T cells which express TCRs comprised of particular Vβs--all other components of the TCR being essentially inconsequential to this interaction. Superantigens, differ from the antigens generally recognized by T cells in two important ways. First the superantigen generally binds to the MHC molecule as an intact protein (not as a peptide) and it binds to a different site than do peptide antigens. Second the T cell response requires the interaction of only the Vβ element of the T cell receptor--all other components of the TCR being essentially inconsequential. This means that the superantigen is able to react with a much larger number of T cells than are peptide antigens.

"Superantigen species" includes "superantigens" or "superantigen derivatives".

"Superantigen derivative" means a molecule whose structure, at least, contains an amino acid sequence substantially identical to an amino acid sequence presented by a superantigen or portions of a superantigen required for binding to

either the MHC or the T cell. It includes both mutated and modified superantigen derivatives.

"Mutated superantigen derivative," or "fragment," is defined as a molecule where the actual amino acid sequence of the mutation has been altered relative to the native form of the molecule. The alteration may be a substitution, an addition or a deletion.

"Modified superantigen derivative," or "fragment," is defined as a molecule that contains an amino acid sequence identical to an amino acid sequence of superantigens, but contains modifications other than mutations not found in the superantigen molecule itself. Examples would include additions of carbohydrate or lipid moieties.

"Cytotoxic agent" means any natural or synthetic substance having the ability to kill cells or having a destructive action upon cells, for example, methotrexate, etc. It specifically includes substances that have a predilection for activated T cells. Activation of T cells leads to several phenotypic changes which may render them more susceptible to cytotoxic agents. The most obvious examples of such phenotypic changes include the state of rapid cell division and the expression or up-regulation of surface molecules such as Fas. Rapidly dividing cells are more susceptible to a number of cytotoxic agents and the expression of Fas makes the cells susceptible to killing by Fas ligand.

"Immunosuppressive agent" means any natural or synthetic substance having the ability to reduce, for example, the response to autoimmune disease, an immune response by way of interfering with lymphocyte growth. In general, immunosuppressive agents can include antimetabolites, irradiation or antibodies.

"Subpopulation" means a subset of T cells as defined by a specific Vβ region. Specific superantigens interact with T cells expressing particular Vβ elements. T cells expressing a particular Vβ elements are known in the art as a T cell subpopulation.

The present invention is embodied in a method for the co-administration of a superantigen with one or more cytotoxic agents to modify the responsiveness of pathogenic subsets of T cells with minimal accompanying release of potentially toxic immune mediators in the prevention or treatment of, for example, autoimmune diseases. In the method of the preferred embodiment Staphylococcal enterotoxin B (SEB), which engages Vβ3, 12, 14, 15 and 17 TCRs that have been implicated in rheumatoid arthritis, is administered in conjunction with methotrexate and dexamethasone. Methotrexate is commonly used in rheumatoid arthritis though its precise mechanism of action is unknown. It is demonstrated, in Example 3 and Fig. 6, *in vitro* methotrexate administration in combination with SEB prevents the generation of T cell blasts and leads to the disappearance of most Vβ3, 12, 14 and 17 cells in human peripheral blood leukocytes (hPBLs). However, interleukin 2 (IL-2) as well as other cytokines are still produced. In order to reduce the production of IL-2 and tumor necrosis factor alpha (TNFα), an immunosuppressive agent, for example, dexamethasone, is added to the regimen.

Initially, intact SEB is used in the treatment of rheumatoid arthritis. Obviously, since this superantigen leads to the elimination of T cells in addition to Vβ3, 12, 14, 15 and 17 cells (namely Vβ20), it is preferable to narrow its specificity. A more significant concern is the possibility of even more limited TCR Vβ involvement in rheumatoid arthritis. Kappler *et al.* ((1992) J. Exp. Med. 175:387) have shown that certain mutations in SEB alter its specificity in mice. To enable the refinement of the specificity to include only requisite Vβs, further investigations concerning these mutants versus human TCRs are necessary.

The goal of the instant invention is the elimination of subsets of T cells which include pathogenic T cells, without unduly incapacitating the overall immune system or causing unacceptable toxicity. The dose of methotrexate is expected to be well tolerated by most subjects, particularly, as only a single dose

may be required. Preliminary experiments show methotrexate does not prevent the release of IL-2. Even though this and other cyt kines play a beneficial role in immune responses, their release in large quantities is potentially toxic. An example of this toxicity is that which occurs in Toxic Shock Syndrome.

The therapeutic composition of the present invention is preferably administered parenterally by injection or by continuous infusion by an implanted pump. Also, other effective administration forms, for example, parenteral slow-release formulations, are also envisioned.. The preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used.

Regardless of the manner of administration, the specific dose is calculated according to the approximate body weight of the patient. Further, the refinement of calculations necessary to determine the appropriate dosage for treatment involving each of the formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them without undue experimentation.

The following examples serve to explain and illustrate the present invention. Said examples are not to be construed as limiting of the invention in anyway. Various modifications are possible within the scope of the invention.

Example 1. Development of an *In Vitro* System to Study Superantigen Effect on Human T cells

Several points are demonstrated by the study of superantigen effects on human T cells. First, superantigens are active at extremely low levels. Next, flow cytometric analysis of superantigen stimulated human peripheral blood lymphocytes can be used to quantitate the effect on subsets of T cells. This analysis permits the selective examination of the cells which are stimulated, those cells which are unaffected, $CD4^+$ T cells, and examination of Vβ defined

subpopulations of T cells. Finally, short term culture of human peripheral blood lymphocytes does not selectively effect Vβ defined subpopulations of T cells, and thus, can be used to study superantigen mediated effects.

Superantigen induced proliferation of human peripheral blood lymphocytes

Superantigens induce human peripheral blood lymphocytes (hPBLs), containing both T cells and antigen presenting cells, to proliferate at very low concentrations. This stimulation requires the presence of antigen presenting cells bearing MHC class II molecules (e.g., monocytes, dendritic cells and macrophages) and is mediated through the variable portion of the TCR β-chain. The amount of proliferation is quantitated by measuring the amount of tritiated (^{3}H) thymidine incorporated into newly synthesized DNA following a three day stimulation.

Venous blood is obtained from human subjects and the mononuclear cells (hPBLs) are isolated by centrifugation over ficol using standard techniques. 10^5 hPBLs are incubated in round bottom 96 well plates in a final volume of 100 µl of IMDM containing 10 % fetal calf serum and the indicated molar concentration of Staphylococcal enterotoxin B (Sigma, St. Louis, MO) or toxic shock syndrome toxin (Toxin Technologies, Sarasota, FL) for three days in a humidified atmosphere of 10 % CO at 37° C.

Cultures are then pulsed with 0.5 µCi ^{3}H-thymidine (Amersham, Arlington Heights, IL) and culture is continued for 16 hours. Next, the contents of each well are transferred to glass wool filters and unincorporated ^{3}H-thymidine is washed away. In order to quantitate the amount of proliferation (CPM) in each culture, the amount of ^{3}H-thymidine incorporated by the hPBLs, and thus, bound to the filters, is counted by standard liquid scintillation counting techniques; the results of which are shown in Fig. 1. It is shown that both SEB and TSST-1 are extremely potent, stimulating proliferation at concentrations below 10^{-12} M.

Flow cytometric analysis

While extremely sensitive, proliferation assays do not demonstrate that it is the T cells that are responding to the SAgs, nor do they allow the conclusion to be reached that the T cell stimulation is Vβ specific. For these purposes, hPBLs are stained with fluorochrome-conjugated monoclonal antibodies specific for various cell-surface molecules and are examined by flow cytometry.

By using flow cytometry, it is possible to distinguish a population of large cells, "blasts", resulting from superantigen stimulation. Fig. 2 shows the light scatter characteristics of hPBLs that have been cultured *in vitro* for three days. The Y axis shows the ability of the cells to scatter light in the forward direction. This ability directly correlates with the size of the cells. The X axis shows the ability of cells to scatter light at a 90° angle and correlates with the cells' granularity.

Three populations of cells are distinguished on the basis of these two characteristics. Bitmap A is where the small resting B and T cells are found; bitmap C represents the dead and dying cells; and bitmap B is where T cell blasts are found. In panel A there is no population of blast cells, however there is a poorly defined population of cells to the right of B. These cells are shown, using monoclonal antibodies, to be primarily monocytes. Panel B shows the emergence of a large population of T cell blasts in region B following stimulation with SEB. Thus, using only the light scatter characteristics of hPBLs, it is possible to distinguish a separate blast population and a population of small resting T cells. By examining, separately, the blast population and the population of small resting T cells, it is possible to demonstrate that T cells expressing particular Vβs are selectively enriched in the blast population and depleted from the resting population. Because neither the resting cell population nor the blast population are completely pure and because $CD4^+$ T cells respond moderately better to superantigen stimulation, two-color analysis using fluorochrome labeled antibodies

to CD4 and to specific Vβs is routinely performed. Fig. 3 shows the results of such an analysis.

Human peripheral blood lymphocytes are stimulated *in vitro* for 3 days with 1 ng/ml of SEB (3.3×10^{-12} M) and stained with phycoerythrin conjugated monoclonal antibody to CD4 (DAKO, Carpinteria, CA) and with FITC conjugated monoclonal antibody to Vβ3 (T Cell Diagnostics, Cambridge, MA) and analyzed on a Coulter flow cytometer. In Fig. 3, the panel on the left shows the light scatter profile of the resulting cells. Bitmaps A, B, and C encircle the populations of resting cells, proliferating cells (blasts), and dying cells respectively. The lower right panel shows an analysis of the resting cells. The $CD4^+$ cells fall in quadrants 1 and 2 while the Vβ3 cells fall in quadrants 2 and 4. Thus, by dividing the number of cells in quadrant 2 by the combined total of cells in quadrants 1 and 2, it is possible to determine that Vβ3 cells comprise 4.1 % of the $CD4^+$ cells. Similarly, in the upper panel Vβ3 cells are shown to comprise 30.5 % of the blast cells.

Determination of Cell Viability

In vitro assays of superantigen activity take several days. Therefore, a preliminary analysis to determine that culture alone will not lead to changes in Vβ expression by T cells is performed. hPBLs are cultured at 10^6/ml for the number of days indicated in Fig. 4 and each aliquot analyzed by flow cytometry. Cell viability is determined by the number of cells per ml that fall in the A and B bitmaps described in Example 2 and for Vβ3 (T Cell Diagnostics, Cambridge, MA) and phycoerythrin conjugated anti-CD4 (DAKO, Carpinteria, CA). Cell viability falls off immediately to about 65 % at day 1 through 3. Cell viability then drops to about 30 % by day 5, where it remains until day 9. To insure that this cell death is random, the percent of Vβ3 T cells among the resting $CD4^+$ T cells is examined. This percentage remains constant at about 8 % over the entire 9 day experiment.

Accordingly, this assay can be used to examine the effects of superantigens on human T cells in culture.

Example 2. Superantigen Mediated Deletion of Vβ defined Subpopulations of T cells

Previous results (McCormick *et al.* (1993) J. Immunol. 150:3785) show that repeated low doses of SEA lead to the deletion of murine Vβ8 T cells without any demonstrable proliferation. Thus, the effects of dose in the present assay are examined first, showing that substimulatory doses of SEB have no effect on the percent of Vβ 3/CD4 cells in the population of resting T cells in human PBLs.

An alternative approach is to subvert what is normally a stimulatory effect on T cells in order to induce the cells to suicide. This is analogous to current therapy for autoimmune and neoplastic diseases that exploit the rapid proliferation of pathogenic cells in order to render them more susceptible to toxic agents. In rheumatoid arthritis, for example, methotrexate (MTX) is a frequently used drug. High dose methotrexate works by inhibiting folate metabolism, and thus, DNA synthesis. The mechanism of low dose methotrexate, as used in rheumatoid arthritis, is unknown, but there is evidence to support that it may involve interference with adenosine metabolism (Kremer (1994) J. Rheumatol. 21:1).

The effect of methotrexate on the emergence of a population of T cell blasts following a 3 day *in vitro* culture of hPBLs in the presence of SEB is illustrated in Fig. 5. hPBLs are cultured at 10^6/ml either alone, in the presence of 1 ng/ml SEB, or in the presence of 1 ng/ml SEB + 0.1 μg/ml methotrexate. The left and center panels in Fig. 5 reiterate the findings shown above in Fig. 3. That is, culture with SEB leads to the appearance in bitmap B of a distinct population of blasts. The addition of methotrexate to this regimen nearly abolishes this blast

population (right panel) while leading to the presence of more dead and dying cells in bitmap C.

The use of SEB + MTX + dexamethasone to delete Vβ defined subpopulations of T cells from the resting T cells, while preventing the appearance of T cell blasts and minimizing the production of cytokines is shown in Fig. 6. The rationale for including dexamethasone -- as described more fully in Example 3 -- is to prevent cytokine release. Two-color flow cytometric analysis of hPBL was performed following *in vitro* culture under the following conditions: PBL alone, hPBL cultured at 10^6/ml in IMDM plus 10% fetal bovine serum for 4 days; SEB alone, SEB added on day 1 at 1 ng/ml; and SEB+MTX+DEX, MTX (0.1 μg/ml) (Sigma, St. Louis, MO) and DEX (100 μg/ml) (Gensia (Elkins-Sinn), Irvine, CA) are added on day 0 and SEB (1 ng/ml) is added on day 1. On day 4 all cells are stained with anti-Vβ2, 3, 12 and 17 and anti-CD4 as described in Example 3. The results for PBLs from 2 subjects are shown in Fig. 6. In all cases the results are expressed as the percent of $CD4^+$ T cells which express a given Vβ. Vβ2 expressing T cells are not stimulated by SEB and are included in the instant protocol as a control.

In both subjects, it is seen that the percent of Vβ2 resting and total T cells actually increases slightly upon SEB stimulation with or without the addition of MTX and DEX. This is to be expected and reflects the loss from these populations of the SEB stimulated Vβ3, 12, 14 and 17 T cells. Conversely, the percent of Vβ3, 12 and 17 resting T cells decreases with SEB stimulation and SEB + MTX + DEX stimulation. As expected, the percent of Vβ3, 12 and 17 T cells is increased in the blast population, together accounting for 40-50 % of the total blasts. Less than 100 % is indicative of the fact that Vβ14, 15 and 20 expressing T cells are also stimulated by SEB. The increase in the blast population, even in the presence of SEB + MTX + DEX, is a true increase; even though, there are very few blasts. The overall effect is best seen by comparing th percent of T cells

expressing a given Vβ in the unstimulated resting or total T cell population with that in the SEB + MTX + DEX stimulated total T cell population. Through the use of a now available monoclonal antibody specific for human Vβ14 T cells (AMAC, Westbrook, ME), it has been confirmed that Vβ14 T cells can also be deleted using this regimen of SEB + MTX + DEX (data not shown).

A dose-response titration for the ability of MTX to prevent the appearance of T cell blasts in the presence of 1 ng/ml SEB is shown in Fig. 7. The experiment is performed as described above by culturing hPBLs for 3 days in the presence of SEB and MTX and determining the number of T cell blasts per 10,000 resting cells by flow cytometry using light scatter profiles. The experiment is conducted either without (W/O) or with (W) dexamethasone (100 μg/l) present. It is seen that a dose of 0.1 μg/ml of MTX is sufficient to decrease the blasts to background levels, corresponding to a concentration of 2.2×10^{-7} M – a concentration that compares with serum levels in rheumatoid arthritis patients following conventional low dose MTX therapy (Kremer et al. (1986) Arthritis and Rheumatology 29:832).

The use of MTX + DEX in combination with a superantigen to achieve deletion of T cells is not unique to SEB, as shown in Fig. 8. The same analysis as described above is performed using TSST-1(Toxin Technologies, Sarasota, FL), specifically deleting Vβ2 T cells.

Example 3. Inhibition of Superantigen Mediated Cytokine Release

Because superantigens stimulate a much larger percentage of total T cells than do conventional antigens, they also stimulate the release of much larger amounts of cytokines. While these cytokines play an essential role in the response to conventional antigens, superantigens stimulate the release of toxic levels. Preliminary experiments demonstrate that although the addition of MTX prevents th accumulation of T cell blasts in response to superantigen stimulation, it does

not prevent cytokine release. Furthermore, DEX prevents cytokine release but is not very effective in preventing the accumulation of T cell blasts. Consequently, these two therapeutic agents are combined in the practice of the instant invention. Example 2 demonstrates that DEX does not interfere with the ability of MTX to delete superantigen activated T cells. The following protocol gives rise to data that demonstrate that dexamethasone inhibits superantigen mediated cytokine release *in vitro* and that prevents toxicity in an *in vivo* mouse model.

Human peripheral blood leucocyte culture supernatants are assayed at the times indicated in Fig. 9 for the presence of interleukin 2 (IL-2) and TNFα. One milliliter cultures of 10^6 hPBLs are assayed. Where indicated, SEB (recombinantly-produced at Supragen, Lakewood, CO) is added at 1 ng/ml, MTX is added at 0.1 μg/ml and DEX is added at 100 μg/ml.

IL-2 is measured by assaying culture supernatants for the ability to promote the growth of the IL-2 dependent cell line HT-2 as described in Kappler *et al.* ((1981) J. Exp. Med. 153:1198). Briefly, 4000 HT-2 cells were added to 2-fold dilution's of culture supernatant in a 96 well plate with a final volume of 100 μl of dumdum plus 10 % fetal bovine serum. Following overnight culture HT-2 cell viability is assessed visually and then quantitated by the MTT assay (Mosmann (1983) J. Immunol. Meth. 65:55). The minimal amount of IL-2 required to maintain greater than 90 % survival of the HT-2 cells is 10 units. Levels of TNFα (R & D Systems, Minneapolis, MN) are determined by ELISA following the manufacturers instructions.

Only supernatants from cultures with SEB alone or SEB + MTX contain significant amounts of IL-2 or TNF as demonstrated in Fig. 9. In fact, the addition of DEX almost completely abolishes the production of these two cytokines. The concentration of DEX used, while high, can be achieved *in vivo* and because it need not be given chronically is expected to have acceptable toxicity.

An established animal model is used to examine the protective effects of DEX *in vivo* -- that of d-galactosamine (d-Gal) enhanced superantigen toxicity murine model (Miethke *et al.* (1992) J. Exp. Med. 175:91). Although the precise mechanism of the toxicity is not understood, it has been demonstrated using antibody to TNF that TNF is critical. Miethke *et al.* (1993) Eur. J. Immunol. 23:1494. B10.BR mice (Jackson Laboratories, Bar Harbor, ME) are injected with 10 mg SEA, 30 mg d-galactosamine (Sigma, St. Louis , MO) or a combination of both intramuscularly (I.M.) in phosphate buffered saline (PBS). Staphylococcal enterotoxin A (SEA) is used in this experiment due to its greater toxicity in mice than SEB. Concurrently, dexamethasone in PBS, is injected intraperitoneally (I.P.). The animals are then examined at 16 and 24 hours for signs of toxicity, i.e., death. Fig. 10 shows that while neither SEA nor dGal alone are toxic, of the 19 mice that received both SEA and dGal, all 19 were dead by 16 hours. The administration of 10 μg of DEX is sufficient to protect 4 out of 4 mice from such toxicity. Moreover, the administration of 1 μg DEX gave partial protection. Only 2 of the 7 mice treated in this manner died during the course of the experiment. Mice that survive the first 24 hours generally show no subsequent toxicity.

Example 4. A System to Test the Method in Rhesus Macaques

In order to test superantigen mediated deletion of T cells in a primate model, experiments in monkeys have begun. Fig. 11 shows a preliminary experiment using PBLs from a Rhesus Macaque (PBLs provided by Dr. Peter Didier, Tulane Regional Primate Center, Covington, LA). The experiment is carried out as described for human PBLs. Briefly, monkey PBLs are isolated over ficol and put into culture at 10^6/ml in IMDM plus 10 % FCS for three days. Where indicated in the figure, SEB is added at 0.1 ng/ml and MTX at 0.1 μg/ml. This preliminary experiment demonstrates several critical features.

First, it has been found that some commercially available monoclonal antibodies to human Vβ elements (anti-Vβ3, T Cell Diagnostics, Cambridge, MA, and anti-Vβ 17, AMAC, Westbook, ME) and to human CD4 molecules (American Type Culture Collection (OKT4), Rockville, MD) cross-react on primate cells. More importantly, antibodies to primate TCR Vβ elements homologous to human elements that respond to SEB – namely Vβ 3 and Vβ17 – cross-react with Rhesus T cells. Next, this analysis shows that, as expected, Vβ3 Rhesus cells are, indeed, stimulated by SEB. Finally, as is true for human PBLs stimulated with SEB + MTX ,Vβ3 cells are largely eliminated from the resting T cell population and T cell blasts are completely eliminated. Thus, the reagents are available to confirm the efficacy of the SEB + MTX + DEX regimen to delete T cells in a primate system.

The foregoing description of the invention is exemplary for purposes of illustration and explanation. Without departing from the spirit and scope of this invention, one skilled in the art can make various changes and modifications to the invention to adapt it to various usages and conditions. As such, these changes and modifications are properly, equitably, and intended to be within the full range of equivalence of the following claims.

TABLE I

KNOWN SUPERANTIGEN SEQUENCES AND STRUCTURES

Staphylococcus	Staphylococcal enterotoxin A	Huang *et al.* (1987) J. Biol. Chem. 262:7006; Betley *et al.* (1988) J. Bacteriol. 170:34
	Staphylococcal enterotoxin B	Jones & Khan (1986) J. Bacteriol. 166:29; Huang & Nergdoll (1970) J. Biol. Chem. 245:3510; Hanelli *et al.* (1985) Proc. Natl. Acad. Sci. 82:5850
	Staphylococcal enterotoxin C1 and C3	Schmidt & Spero (1983) J. Biol. Chem. 258:6300; Bohach & Schlievert (1987) Mol. Gen. Genet. 209:15; Couch & Betley (1989) J. Bacteriol. 171:4507
	Staphylococcal enterotoxin D	Bayles & Iandolo (1988) J. Bacteriol. 171:4799
	Staphylococcal enterotoxin E	Couch *et al.* (1989) J. Bacteriol. 17:2954
Toxic Shock Toxin		Schlievert *et al.* (1981) J. Infect. Dis. 143:509; Blomster-Hautamaa *et al.* (1986) J. Biol. Chem. 261:15783; Bergdoll *et al.* (1981) Lancet 1:1017
Exfoliating Toxins		Lee *et al.* (1987) J. Bacteriol. 169:3904
Staphylococcus	Staphylococcal pyrogenic toxin C	Goshorn & Schlievert (1988) Infect. Immun. 56:2518; Tomal *et al.* (1990) J. Exp. Med. 172:359
Mouse Mammary Tumor Virus		Fasel *et al.* (1982) EMBO J. 1:3; Donehower *et al.* (1981) J. Virol. 37:226; Donehower *et al.* (1983) J. Virol. 51:941; Racavlakin & Prakaah (1984) J. Virol. 45:604; Choi *et al.* (1991) Nature 350:203; Acha-Orbea *et al.* (1991) Nature 350:207; Pullen *et al.* (1992) J. Exp. Med. 175:41; Moore *et al.* (1987) J. of Virology 61:400

WE CLAIM:

1. A method for the treatment of patients suffering from diseases mediated by T cells having a limited Vβ profile comprising coadministering to said patient a therapeutically effective amount of a superantigen or superantigen derivative and pharmacological agent or agents in a pharmaceutically acceptable carrier, wherein said superantigen or superantigen derivative specifically targets the pathogenic Vβ expressing T cells for subsequent inactivation or deletion by said pharmacological agent or agents.

2. The method of claim 1, wherein said superantigen is a bacterial superantigen.

3. The bacterial superantigen of claim 2, wherein said bacterial superantigen is a Staphylococcal enterotoxin or toxic shock syndrome toxin.

4. The bacterial superantigen of claim 2, wherein said bacterial superantigen is Staphylococcal enterotoxin B (SEB).

5. The method of claim 1, wherein said superantigen derivative is a modified superantigen fragment.

6. The method of claim 1, wherein said superantigen derivative is a mutated superantigen or mutated superantigen fragment.

7. The superantigen derivative of claim 5, wherein said superantigen derivative is a bacterial superantigen derivative.

8. The superantigen derivative of claim 6, wherein said superantigen derivative is a bacterial superantigen derivative.

9. The bacterial superantigen derivative of claim 7, wherein said bacterial superantigen derivative is a Staphylococcal enterotoxin derivative or toxic shock syndrome toxin derivative.

10. The bacterial superantigen derivative of claim 8, wherein said bacterial superantigen derivative is a Staphylococcal enterotoxin derivative.

11. The bacterial superantigen derivative of claim 7, wherein said bacterial superantigen is a Staphylococcal enterotoxin B (SEB) derivative.

12. The bacterial superantigen derivative of claim 8, wherein said bacterial superantigen is a Staphylococcal enterotoxin B (SEB) derivative.

13. The method of claim 1, wherein at least one of said pharmacological agents is a cytotoxic agent.

14. The cytotoxic agent of claim 6, wherein said cytotoxic agent is methotrexate.

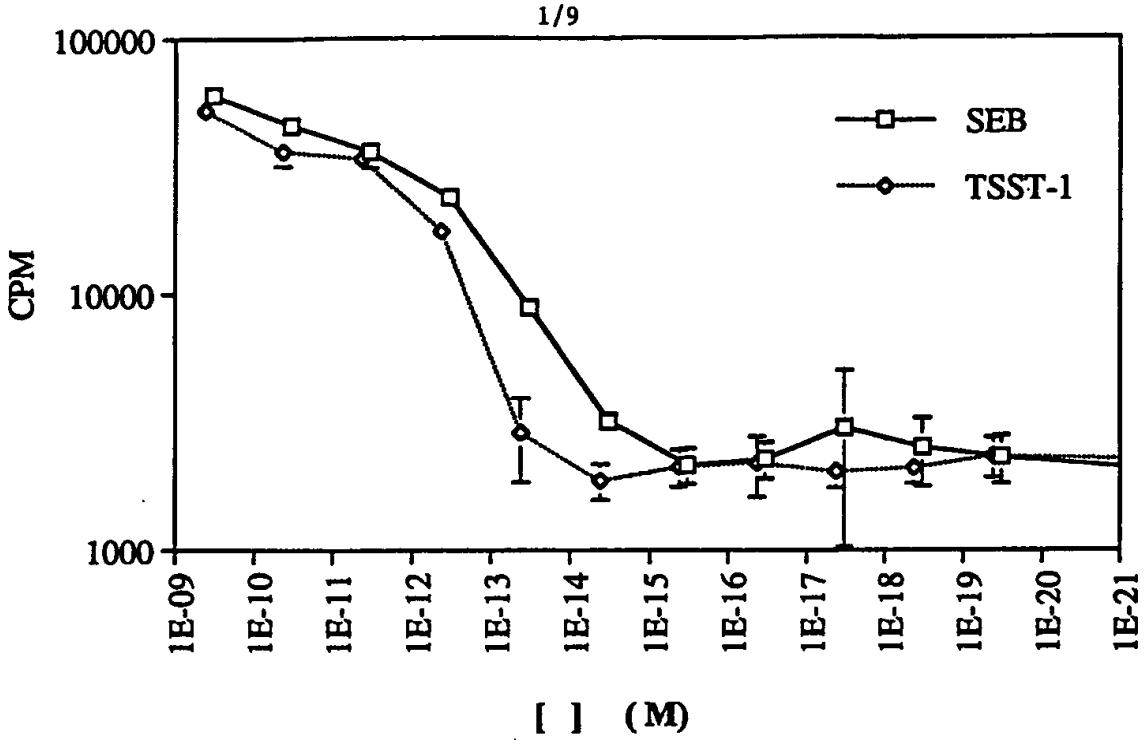
15. The method of claim 1, wherein at least one of said pharmacological agents controls potential cytokine toxicity

16. The method of claim 8, wherein said pharmacological agent is an immunosuppressive.

17. The immunosuppressive agent of claim 9, wherein said immunosuppressive agent is a corticosteroid.

18. The immunosuppressive agent of claim 9, wherein said immunosuppressive agent is dexamethasone.

19. The method of claim 1, wherein at least one subpopulation of T cells of said patient is deleted without activating said T cells to release toxic levels of cytokines.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CPM
SEB
TSST-1
1E-09
1E-10
1E-11
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1E-13
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1E-19
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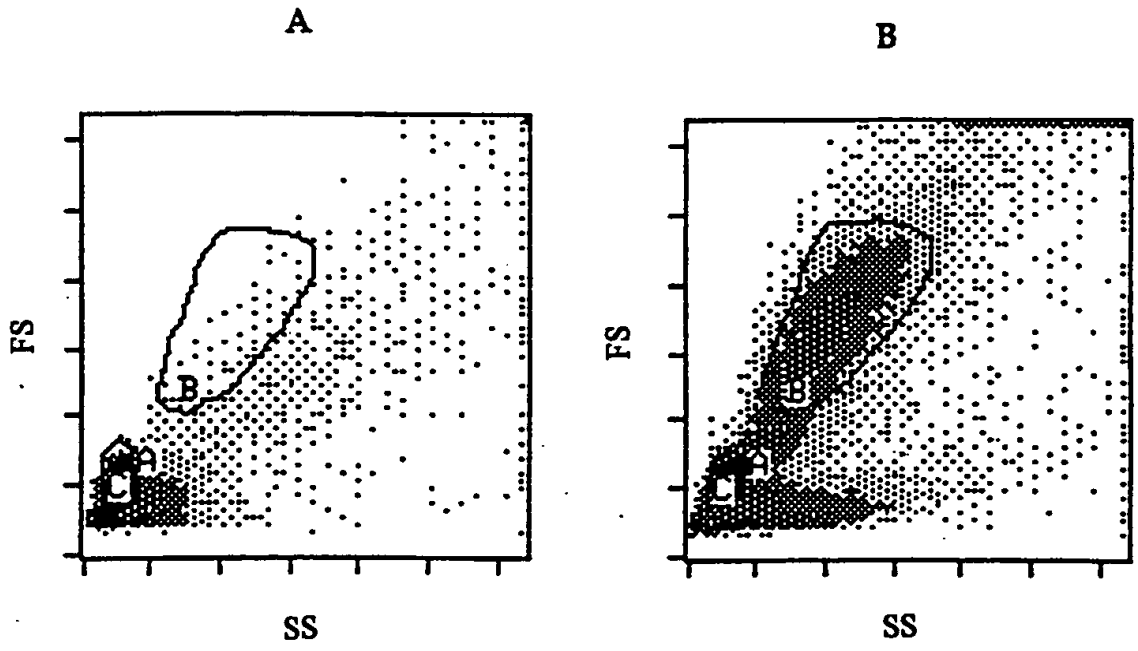
1E-21
[ ] (M)

Fig. 1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B
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SS
FS
SS
B
C
B
C

Fig. 2

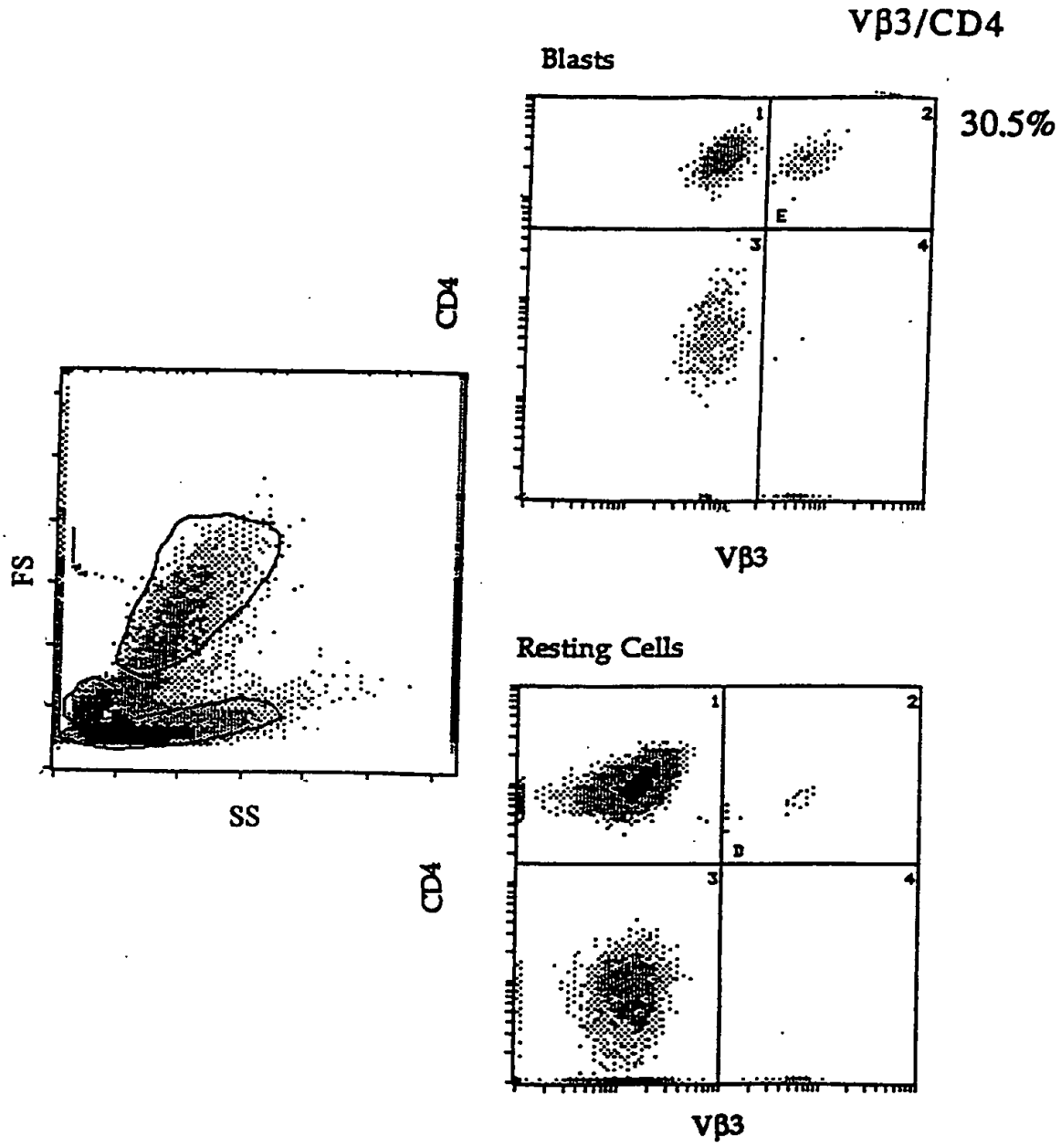

Vβ3/CD4
Blasts
30.5%
1
2
3
4
E
CD4
Vβ3
FS
SS
Resting Cells
1
2
3
4
D
CD4
Vβ3

Fig. 3

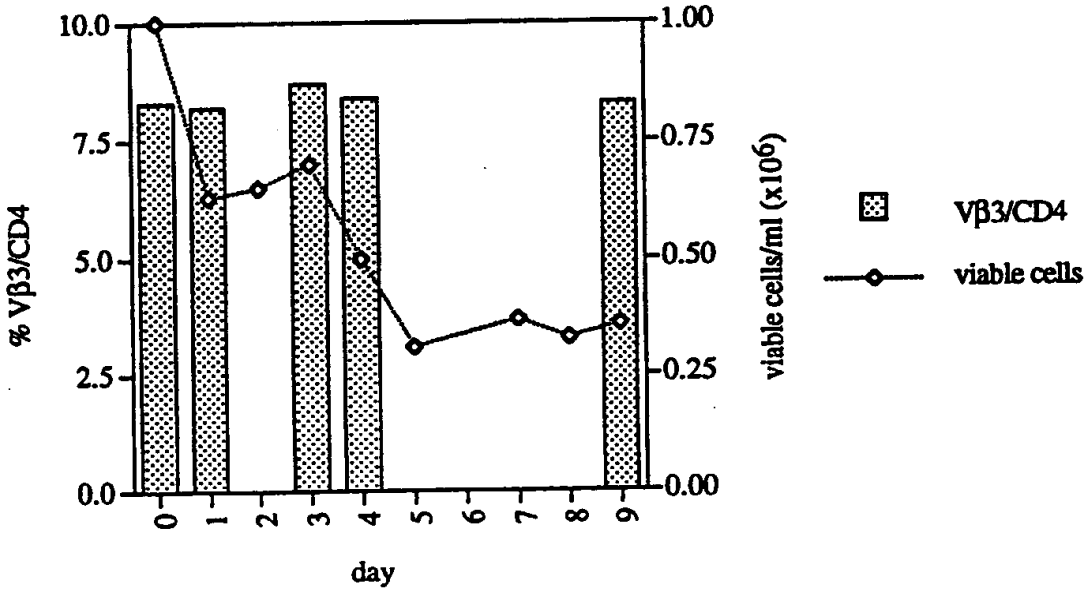

10.0
7.5
5.0
2.5
0.0
% Vβ3/CD4
1.00
0.75
0.50
0.25
0.00
viable cells/ml (x10⁶)
0
1
2
3
4
5
6
7
8
9
day
Vβ3/CD4
viable cells

Fig. 4

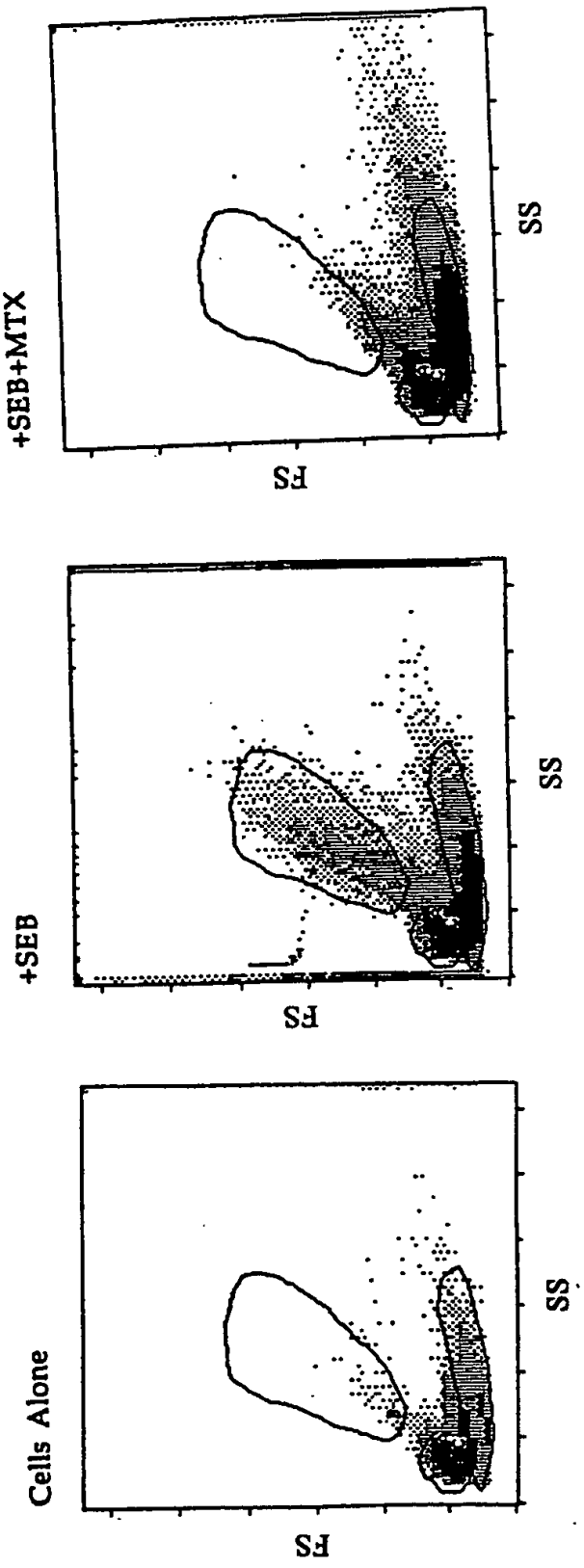


Fig. 5

donor=BT	%V B2 of CD4+			%VB3 of CD4+			%VB12 of CD4+			%VB17 of CD4+		
	resting	blasts	total	resting	blasts	total	resting	blasts	total	resting	blasts	total
no stimulation	10.7%		10.6%	6.9%		7.1%	1.7%		1.7%	4.7%		4.8%
SEB	12.9%	1.2%	9.3%	2.3%	30.5%	7.0%	0.5%	7.0%	2.2%	1.4%	13.8%	6.2%
SEB+MTX/DEX	12.6%	11.9%	12.6%	1.1%	31.5%	1.3%	0.5%	3.2%	0.6%	0.6%	13.2%	1.0%

donor=RGK	%V B2 of CD4+			%VB3 of CD4+			%VB12 of CD4+			%VB17 of CD4+		
	resting	blasts	total	resting	blasts	total	resting	blasts	total	resting	blasts	total
no stimulation	9.1%		9.2%	5.5%		5.8%	1.9%		1.9%	4.7%		4.9%
SEB	11.5%	0.9%	6.0%	0.8%	27.9%	4.5%	0.3%	4.9%	1.2%	0.4%	12.4%	6.3%
SEB+MTX/DEX	10.8%	1.4%	9.7%	0.4%	32.8%	1.1%	0.0%	1.5%	0.4%	0.8%	7.9%	1.5%

Fig. 6

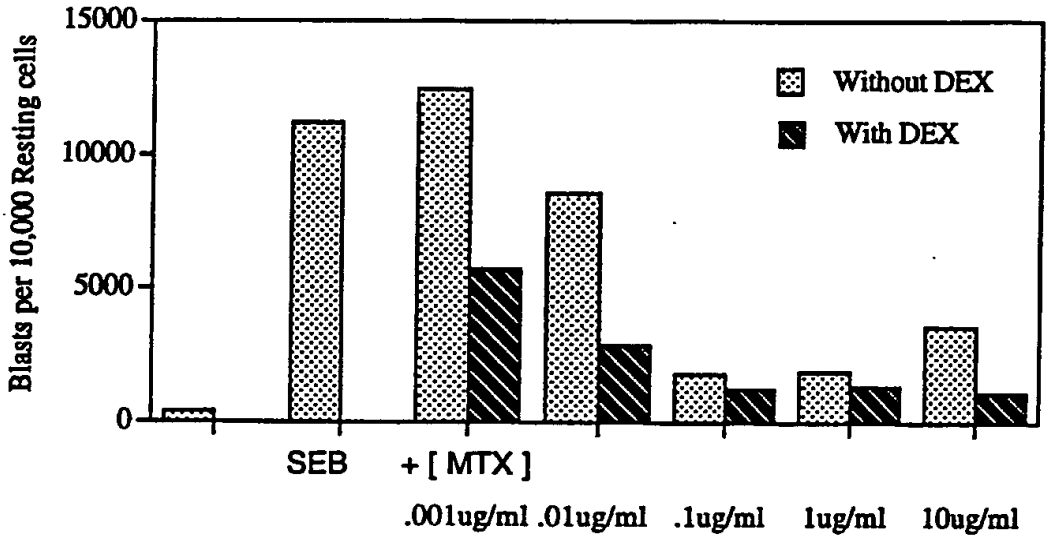


Fig. 7

	resting	Vβ2/CD4 blasts	total
no stimulation	9.3%	--	9.3%
TSST-1	0.2%	37.7%	7.4%
TSST-1 + MTX/DEX	1.0%	39.5%	2.0%

Fig. 8

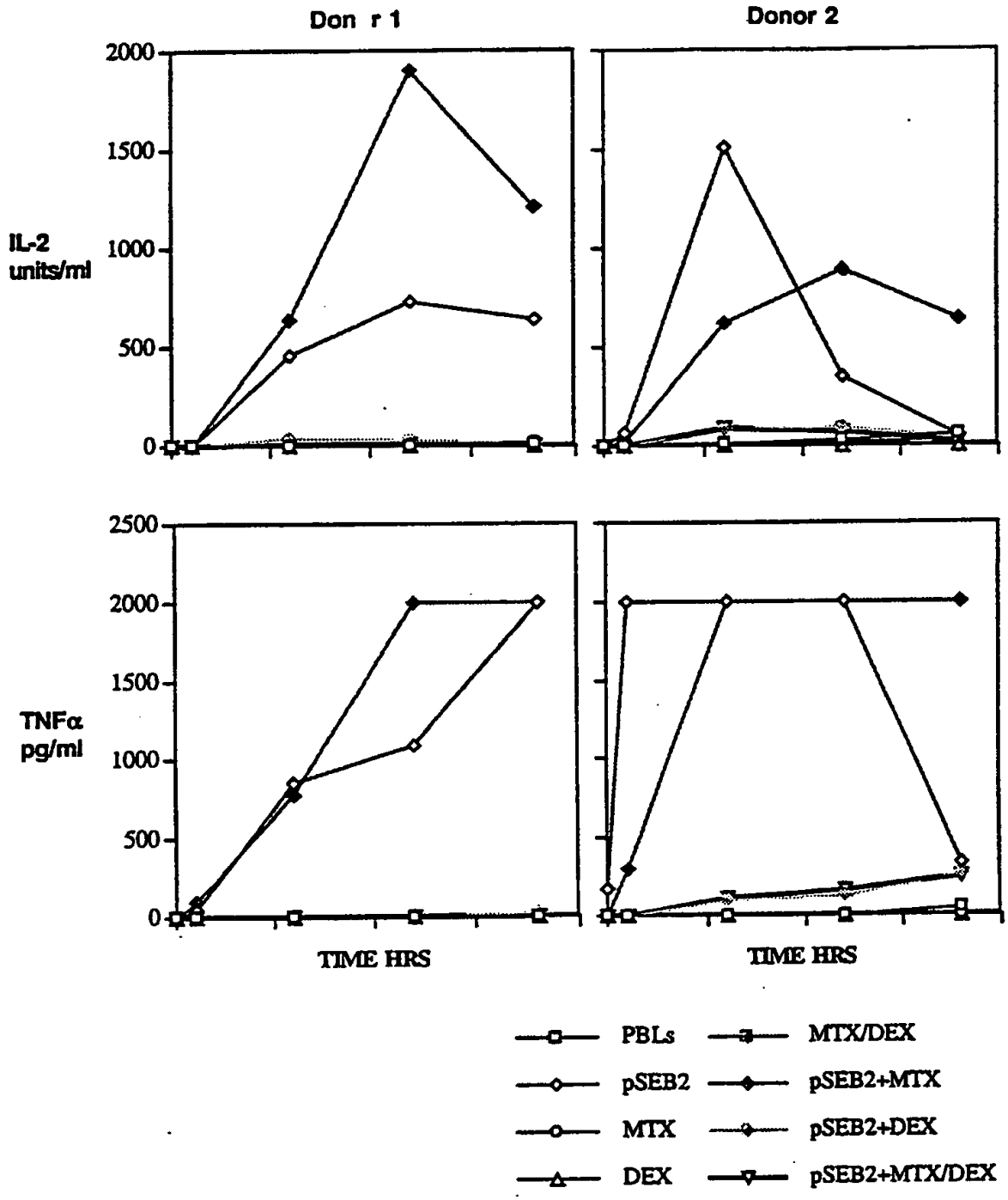
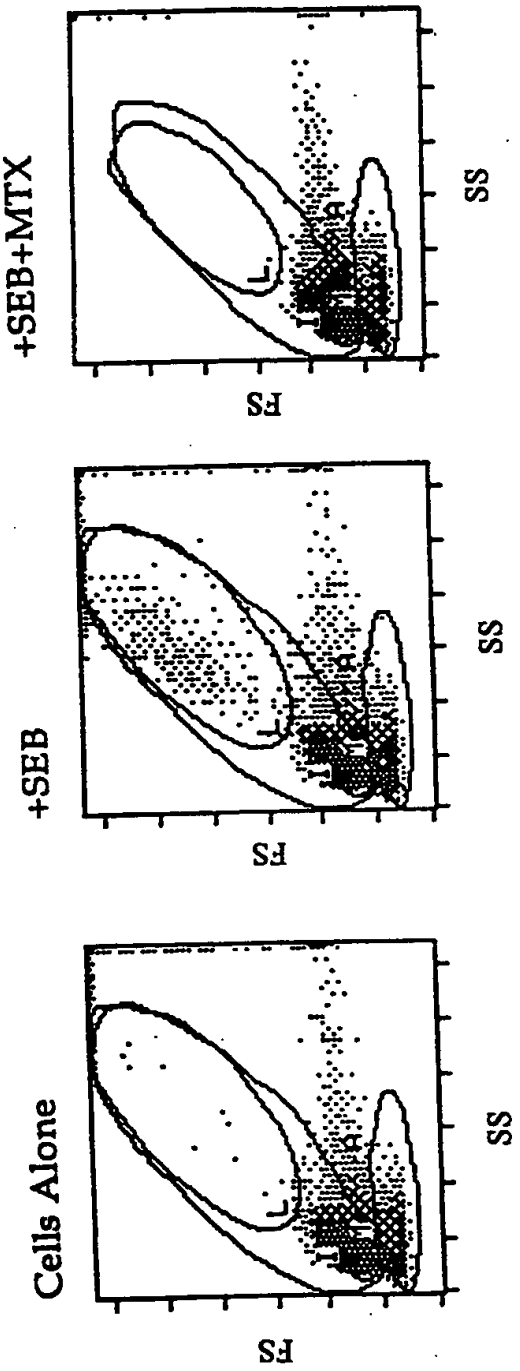

Don r 1
Donor 2
IL-2 units/ml
2000
1500
1000
500
0
TNFα pg/ml
2500
2000
1500
1000
500
0
TIME HRS
TIME HRS
PBLs
MTX/DEX
pSEB2
pSEB2+MTX
MTX
pSEB2+DEX
DEX
pSEB2+MTX/DEX

Fig. 9

Condition	# Dead/Total	
SEA i.m.	0/4	
dGal i.m.	0/4	
dGal+SEA i.m.	19/19	
dGal+SEA+DEX 1mg	0/4	
dGal+SEA+DEX 100ug	0/4	
dGal+SEA+DEX 10ug	0/4	
dGal+SEA+DEX 1ug	2/7	by 24 hours
dGal+SEA+DEX 0.1ug	2/3	by 24 hours
dGal+SEA+DEX 0.01ug	3/3	by 24 hours

Fig. 10

A



B

Vb3/CD4	Resting Cells	Blast Cells	Total
Cells Alone	3.20%	3.14%	3.23%
+SEB	0%	16.80%	4.82%
+MTX	0%	9.54%	0.92%

Fig. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08193

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A 61K 39/02
US CL : 424/184.1, 236.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 236.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	European Journal Immunology, Volume 23, issued 1993, Lussow et al, "Peripheral clonal deletion of superantigen-reactive T cells is enhanced by cortisone", pages 578-581, see entire document.	1-4, 7-13, 16-17
X	European Journal Immunology, Volume 22, issued 1992, Gonzalo et al, "Expansion and clonal deletion of peripheral T cells induced by bacterial superantigen is independent of the interleukin-2 pathway", pages 1007-1011, see entire document.	1-4, 7-12, 16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 1996	Date of mailing of the international search report 16 September 1996 (16.09.96)
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T. WESSENDORF Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08193

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Exp. Med., Volume 176, issued July 1992, Vanier et al, "Cyclosporin A Markedly Enhances Superantigen-induced Peripheral T Cell Deletion and Inhibits Anergy Induction", pages 37-46, see entire document.	1-4, 7-12, 13, 16
Y	Journal of Neuroimmunology, Volume 43, issued 1993, Soos et al, "Treatment of PL/J mice with the superantigen, staphylococcal enterotoxin B, prevents development of experimental allergic encephalomyelitis", pages 39-43, see entire document.	1-19
X	J. Exp. Med. Volume 177, issued May 1993, Gonzalo et al, "Glucocorticoid-mediated Control of the Activation and Clonal Deletion of Peripheral T Cells In Vivo", pages 1239-1246, see entire document.	1-4, 7-13, 19
X	The Journal of Biological Chemistry, Volume 269, No. 51, issued 23 December 1994, Mehindate et al, "Induction of Chemokine Gene Expression by Major Histocompatibility Complex Class II Ligands in Human Fibroblast-like Synoviocytes Differential Regulation by Interleukin-4 and Dexamethasone", pages 32063-32069, see entire document.	1-4, 7-13, 15, 18, 19
X	Cancer Research, Volume 44, issued November 1984, Cesario et al, "Effect of Antineoplastic Agents on Interferon Production in Human Peripheral Blood Mononuclear Cells", pages 4962-4966, see entire document.	1-4, 7-13